

Reprinted from the American Journal of Physiology

VOL. XXXIV — MAY 1, 1914 — No. II

FACTORS AFFECTING THE COAGULATION TIME
OF BLOOD

I. THE GRAPHIC METHOD OF RECORDING COAGULATION USED
IN THESE EXPERIMENTS

BY W. B. CANNON AND W. L. MENDENHALL

[FROM THE LABORATORY OF PHYSIOLOGY IN THE HARVARD MEDICAL SCHOOL]

WELLCOME INSTITUTE LIBRARY	
Coll.	welMOMec
Coll.	pam
No.	WH 310
	1 9 1 4
	C 22 f4



22500893655

FACTORS AFFECTING THE COAGULATION TIME OF BLOOD ¹

I. THE GRAPHIC METHOD OF RECORDING COAGULATION USED IN THESE EXPERIMENTS

BY W. B. CANNON AND W. L. MENDENHALL

[From the Laboratory of Physiology in the Harvard Medical School]

Received for publication March 30, 1914

MANY methods have been devised for determining coagulation time. The description of these methods is unnecessary in this paper, for they have been considered critically in two comparatively recent reviews, one by Addis,² the other by Morawitz.³ With different methods the coagulation time of blood (at 20°) has been set down as ranging from approximately 5 minutes (Addis) to 20 minutes (Morawitz and Bierich). This great discrepancy shows that there is no definite "coagulation time" quite independent of the method used, i.e., the conditions peculiar to any coagulometer are likely to affect the time of clotting. Since to draw blood any instrument is a foreign body, all that is required of an instrument is that the conditions of its use shall be constant.

The conditions defined by Addis as being essential for accurate estimation of coagulation time are as follows:

1. The blood must always be obtained under the same conditions.
2. Estimates must all be made at the same temperature.
3. The blood must always come in contact with the same amount and kind of foreign material.

¹ A preliminary report of these experiments was presented at the meeting of the American Physiological Society, Dec. 29, 1913. See Proceedings, This journal, 1914, xxxiii, p. xxxviii; also p. 372.

² ADDIS: Quarterly journal of experimental physiology, 1908, i, p. 305.

³ MORAWITZ: Abderhalden's Handbuch der biochemischen Arbeitsmethoden, 1911, v, pp. 235-252.

4. The end point must be clear and definite and must always indicate the same degree of coagulation.¹

Besides conforming to these four conditions it seemed to us that the ideal instrument should also yield a permanent objective record, made by the blood itself. The form of coagulometer finally employed is illustrated diagrammatically in Figure 1. It consists essentially of a light aluminum lever² with the long arm nearly counterpoised by a weight W . The long arm is prevented from falling by a support S , and is prevented from rising by a horizontal

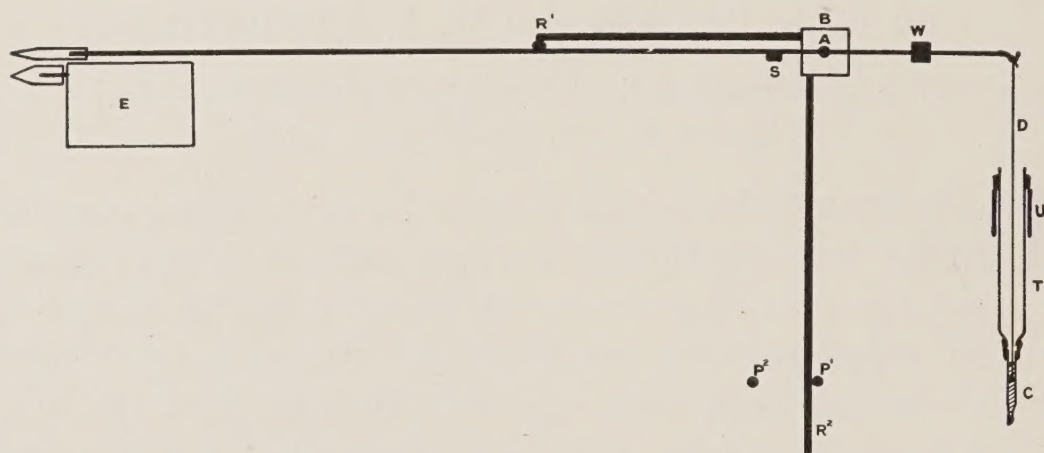


FIGURE 1. Diagram of the graphic coagulometer. The cannula at the right rested in a water bath not shown in this diagram. For further description see text.

right-angled rod reaching over the lever at R^1 and fixed into the block B which turns on the axis A . Into the same block is fixed the vertical rod R^2 . When this rod is moved from the post P^1 , against which it is held by the weight of the horizontal rod R^1 , towards the other post P^2 , the check on the long arm of the lever is lifted, and, if the short arm is heavier, the long arm will then rise.

The cannula C , into which the blood is received, is 2 cm. in total length and slightly more than 2 mm. in internal diameter. It is attached by a short piece of rubber tubing to the tapered glass tube T , 5 cm. long and 5 mm. in internal diameter. The upper end of this tube is surrounded by another piece of rubber which supports the tube when it is slid into the U-shaped support U , fixed directly below the end of the short arm of the lever.

¹ ADDIS: *loc. cit.*, p. 314.

² The "heart lever" made by the Harvard Apparatus Company.

By drawing the cannulas from a single piece of glass tubing and by making the distance from shoulder to upper end about 12 mm., receptacles of fairly uniform capacity are assured. All the dimensions, the reach of the rubber connection over the top of the cannula (2-3 mm.), the distance of the upper rubber ring from the lower end of the glass chamber (4 cm.), etc. — were as nearly standard as possible.

A copper wire *D*, 8 cm. long and 0.6 mm. in diameter, bent above into a hook, and below into a small ring slightly less than 2 mm. in diameter, is hung in a depression at the end of the short arm of the lever. The small ring then rests in the upper part of the cannula (see Figure 1). The weight of the copper wire makes the short arm of the lever heavier than the long arm by 30 mgm., when the delicate writing point is moving over a lightly smoked drum. Half a dozen of these standard wires were needed.

The precautions taken to fulfill the conditions stated above as essential for accurate determination of the coagulation time, were as follows:

1. Drawing the blood. — The blood was taken from the femoral artery. The artery (usually the right) was laid bare in the groin and freed from surrounding tissue. A narrow artery clip with each limb enclosed in soft rubber tubing, and with its spring exerting gentle pressure, was placed on the artery immediately below the deep femoral branch, thus allowing no blood to stagnate above the clip. Between the clip and a ligature applied about 1.5 cm. below, an opening was made. The blood was carefully milked out of the vessel between a blunt dissector moved beneath, and a small forceps, twisted into a pinch of absorbent cotton, moved above.

The cannula, cleaned in water, alcohol, and ether, was set in the rubber connection of the glass tube; the point of the cannula was then lubricated with vaseline, and slipped into the artery. The pressure of the clip on the artery was next very slightly released and blood was allowed to flow into the cannula up to the lower border of the rubber connection. Only a good-sized drop of blood was needed. Sometimes the blood ran one or two millimeters above or below, but without appreciably changing the result. Since the clip was situated on the femoral immediately

below a branch in which the circulation persisted, *the blood received in the cannula was always fresh from the moving stream*. As soon as the clip gripped the artery again, the cannula was slipped out. A helper then promptly milked the vessel in the manner described above, and covered it with a pad of absorbent cotton, smeared with vaseline, to prevent drying. Thereby blood was not permitted to stagnate; and when a new sample was to be taken, the vessel was clean and ready for use.

The tip of the cannula was at once plugged by plunging it into a flat mound of plasticine about 3 mm. high. It was drawn off sidewise lest the plasticine plug be pulled out again. One of the copper wires *D* was now slid into the tube and cannula, the tube slipped into the **U**-support, and the wire lifted and hung on the lever. This procedure, from the moment blood began to flow until the wire was hung, consumed usually about 20 seconds.

2. Uniform temperature.—Under the **U**-support was placed a large water bath, in which the cannula and the tapering part of the tube were submerged. A thermometer was fixed to the **U**-support so that the bulb came near the cannula in the bath. The water was kept within a degree of 25° C. This temperature was chosen for several reasons: (*a*) The cannula has room temperature and rapidly cools the small volume of blood that enters it. To heat blood and cannula to body temperature would take time. A bath near room temperature, therefore, seems preferable to one near body temperature. (*b*) The test of clotting was conveniently made at intervals of a half-minute, and if the clotting process were hastened by higher temperatures, this interval would become relatively less exact. (*c*) A temperature of 25° C. rather than lower was selected because, as Dale and Laidlaw¹ have shown, the coagulation time is much slower for a given change in temperature below 25° than for the same change above. And with slowing of the process the end point, when the determination depends on supporting a weight, is less likely to be sharp. (*d*) The researches undertaken with use of this coagulometer were concerned with factors hastening the process. For that reason and for reason (*b*),

¹ DALE and LAIDLAW: *Journal of pathology and bacteriology*, 1912, xvi, p. 359.

a long rather than a short coagulation time for normal conditions was desirable.

3. Uniformity in the amount and kind of contact with foreign surface. — The capacity of the cannulas was fairly uniform, as stated above; the amount received in them was fairly constant; and the wire hanging in the blood presented approximately the same surface in different observations.

A further condition for insuring consistent treatment of the blood in different cases was that of making the tests for coagulation always at the same intervals. Below the writing point of the lever was set an electromagnetic signal E which recorded half-minutes. At the moment a record was made by the signal (see first signal mark, Figure 2), the clip on the artery was opened, the blood taken, and the process thus begun. In about 20 seconds the cannula was suspended in the water bath, and the wire was hanging on the lever. At the next record by the signal and at every subsequent record the vertical rod R^2 was pushed with the index finger from post P^1 to post P^2 and allowed to move back.¹ This motion was uniform and lasted about one second. The check R^1 on the long arm of the lever was thus raised, and as the wire sank in the blood the writing point rose, recording that coagulation had not taken place (see Figure 2).

4. Definite end-point. — As soon as the blood clotted, the weight of 30 mgm. was supported, and the failure of the lever to rise to the former height in the regular time allowed recorded that the change had occurred.

Very rarely the swing of the lever would be checked for a moment and would then begin to move rapidly, indicating that a strand of fibrin had formed but not sufficiently strong to support the weight, and that when the strand broke, the weight quickly sank in the blood. When this occurred the next record almost always was the short line, which signified that the weight was well supported.

A very slight strand of fibrin was able to prevent the weight

¹ By applying a T-shaped wire to the axis of the second hand of a clock, and bending outward at right angles the ends of the top of the T, it is possible to have the vertical rod R^2 shifted automatically at half-minute intervals. This method was not used extensively in our experiments.

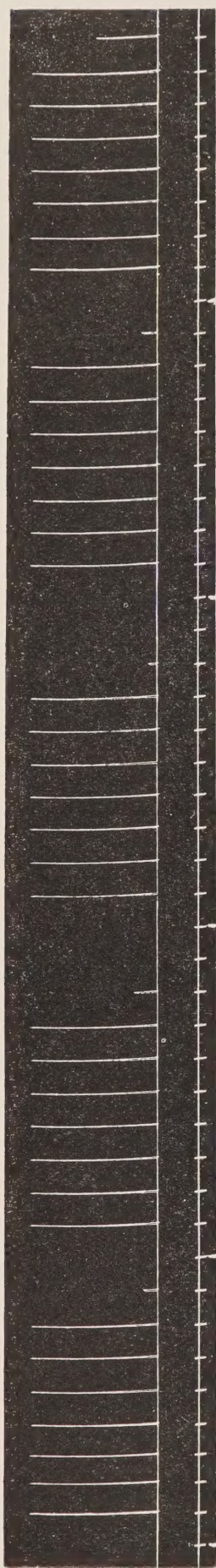


FIGURE 2. Record (reduced one-third) of five successive tests of coagulation, with the animal in a uniform condition. The lower line records intervals of 30 seconds. The marks below the time record indicate the moments when the blood samples were drawn.

from dropping, though at different times the amount of support differed, as shown by the varying length of the final lines (cf. first and last series, Figure 2). These variations are probably a rough indication of the degree of coagulation. In our experiments, however, the length of the final line was disregarded, and merely the fact that the lever failed to swing through its usual distance was taken as evidence of a clot, and the consequent short record was taken as the end point.

As soon as this end point was registered, the tube, wire and cannula were lifted out of the bath; the cannula was then separated from the tube and pulled away from the wire. The clot was thus disclosed, confirming the graphic record.

The method, at least when used at half-minute intervals, did not reveal in all instances the same degree of clotting. Usually, when the process was very rapid, the revealed clot was a thick jelly; whereas, when the process was slow, a strand of fibrin or at most a small amount of jelly was found. This difference in the *degree* of coagulation introduced, of course, an element of inexactness. In our experiments, however, this inexactness was unfavorable to the result we were seeking for, i.e., the acceleration of the process — because the jelly is a later stage than the fibrin strand; and since we nevertheless obtained good evidence of acceleration, we did not in these experiments attempt to determine more accurately differences in the stage of the clotting process.

Cleaning of apparatus.—After the wire was removed from the tube, the clot attached to its ring-tip was carefully brushed away

under cool running water. Under the running water, also, a trimmed feather was introduced into the cannula and the tube to push out the plasticine and to wash out the blood. Wire, cannula and tube were then dropped into a beaker receiving running hot water (about 80° C) and there allowed to remain for about five minutes. On removal from this the parts were shaken free from water, passed through 95 per cent alcohol and again shaken free, passed through ether and let dry.

By having a half-dozen cannulas and wires of standard size, it was possible to save trouble by cleaning a number at one time.

Not infrequently the first few samples of blood taken from an animal showed rapid or somewhat irregular rates of clotting. Some causes for these initial variations will be presented in following papers. The fairly uniform rate of clotting in any individual after the initial stage, varied in 21 different animals from an average of 3 to an average of 10.6 minutes, with a combined average of 5.9 minutes. The conditions for these variations among individuals have not been wholly determined.

The method here described has been employed not only to determine the coagulation time of cat's blood, but also that of human blood taken repeatedly through the skin at the base of the thumb nail. The skin was washed in soap and water, and rinsed in alcohol and ether before each test and then punctured with a sharp, well-pointed, three-cornered needle. The drop of blood that appeared on the skin was drawn into the cannula, and then the procedure was that above described. Following are figures obtained on one occasion:

Coagulation time	Variations from the average
5.5 minutes	+ 0.6 minutes
5 "	+ 0.1 "
4.5 "	- 0.4 "
4.5 "	- 0.4 "
5 "	+ 0.1 "
<u>5</u>	<u>+ 0.1</u>
Average 4.9 minutes	Average error $\pm$ 0.3 minutes
Per cent average error = 6.	

Doubtless if the lever were moved more frequently than every half-minute the average error would be reduced.

THE · PLIMPTON · PRESS · NORWOOD · MASS · U · S · A